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Description

This invention pertains to a sterile composition for treating cancer and genetic diseases, particularly for the suppression or eradication f bone marrow, and to compositions having as their active ingredient a radionuclide complexed with a macrocyclic aminophosphonic acid.

The use of agents which cause partial or total suppression or eradication of the bone marrow has become an accepted part of some procedures used to treat patients with cancers such as leukemias, lymphomas, myelomas and Hodgkin's disease as well as in the treatment of patients suffering from genetic disorders such as sickle cell anemia and thalassemia.

For example, in the treatment of patients having acute lymphoblastic leukemia and acute nonlymphoblastic leukemia, it is sometimes beneficial to employ a therapy regimen which combines chemotherapy using drugs, such as cyclophosphamide, bischloroethyl nitrosourea, cytosine arabinoside, 6-thioguanine and the like, and total body irradiation, followed by bone marrow transplantation.

In situations where the patient is suffering from a genetic disability such as thalassemia or sickle cell anemia, bone marrow transplantation may offer the possibility of a cure. In thalassemia, the afflicted individual has a genetic disorder causing the production of an abnormal hemoglobin and is only able to survive by repeated blood transfusions. Nonetheless, children afflicted with thalassemia major rarely survive to adulthood. In sickle cell anemia, the individual produces an abnormal hemoglobin (i.e., hemoglobin S). The individual homozygous for hemoglobin S has red blood cells that assume a sickle shape at ordinary oxygen tensions. These sickled red blood cells encounter mechanical difficulties in moving through small blood vessels which can lead to thromboses and tissue anoxia.

The use of radionuclides for bone marrow suppression with a phosphonic acid ligand is discussed in published European patent application 291,605 where the use of Sm-153, Gd-159, or Ho-166 complexed with a ligand selected from ethylenediaminetetramethylenephosphonic acid (EDTMP), diethylenetriaminepentamethylenephosphonic acid (DTPMP), hydroxyethylethylenediaminetrimethylenephosphonic acid (HEEDTMP), nitrilotrimethylenephosphonic acid (NTMP), or tris(2-aminoethyl)aminehexamethylenephosphonic acid (TTHMP) is disclosed.

EP-A-0 164 843 describes a complex of particle emitting radionuclides which are complexed with phosphonic acid derivative of organic amine or substituted organic amine compounds wherein the nitrogen and phosphorous are interconnected by an alkalene group. These complexes are described as being useful in the treatment of calcific tumors.

Bone marrow transplantation offers the possibility of eradicating the afflicted individual's defective bone marrow and replacing it with a normal, non-pathogenic, bone marrow. If the abnormal bone marrow of an individual suffering from sickle cell anemia or thalassemia can be eradicated and then replaced with a bon marrow which takes and is reproduced and capable of producing normal hemoglobin, the individual may be cured.

For those situations where bone marrow transplantation can aid in therapy or cure, it would be desirable to have a means of selectively suppressing the bone marrow independent of or with limited total body irradiation.

The present invention is directed to a sterile composition for suppressing bone marrow in a mammal comprising at least one radionuclide selected from Samarium-153, Gadolinium-159, Holmium-166 or Yttrium-90 complexed with at least one macrocyclic aminophosphonic acid ligand containing the 1,4,7,10-tetraazacyclododecane moiety or a physiologically-acceptable salt thereof, wherein the phosphorous of said ligand is attached to the amine nitrogen through an alkylene moiety and wherein the radionuclide in dosage form is present in an amount containing from 18 to 1850 megabecquerels per kilogram of body weight of said mammal, preferrably from 185 to 1850 megabecquerels per kilogram of body weight of said mammal. The method of bone marrow suppression described herein may be used in combination with chemotherapeutic drugs and/or external radiation.

The present invention has significant benefits in that it permits selective bone marrow suppression, that is, the bone marrow can be suppressed with only minimal damage to non-target soft tissues, for example, liver and kidney. Selective bone marrow suppression offers the opportunity to pursue particular treatment regimens which would otherwis be unavailable due to the concerns of exc ssive non-target soft tissue damage, for example, when total body irradiati n is the s l or primary means of obtaining bone marrow suppression. Using th present invention for obtaining bone marrow suppression reduces the risk to the pati nt since the damage to non-target soft tissu is significantly reduced thereby prom ting th general health of the patient and enhancing the prospect of th patient's recovery.

In the composition of the invention, th pref rred macrocyclic aminophosphonic acid moi ty is 1,4,7,10-tetraazacyclododecanetetramethylene-phosphonic acid (DOTMP). The pr sent inv ntion includes the us of

the bone marrow suppressing composition in combination with other drugs and/or radiation sources.

In addition the present invention also includes formulations having a pharmaceutically acceptable carrier, xcipient or vehicl for the composition of th invention.

The methods for preparing such formulations are well known. The formulations are sterile and may be in the form of a susp nsion, injectable solution or oth r suitable pharmaceutically acceptable formulations. Physiologically acceptable suspending media, with or without adjuvants, may be used.

The present invention contemplates the use of one or more other agents or treatments which assist in obtaining bone marrow suppression when used in conjunction with the bone marrow suppressing radionuclide compositions described herein.

With the composition of the present invention the suppression of bone marrow can be achieved by administering to a mammal a bone marrow suppressing amount of said composition. The present invention has significant benefits in that it permits selective bone marrow suppression (the bone marrow can be suppressed with only minimal damage to non-target soft tissues, for example, liver) without the need for large amounts of excess chelant. As will be more fully discussed later herein, the properties of the radionuclide, and of the radionuclide-macrocyclic aminophosphonic acid complex are important considerations in determining which radionuclide composition should be employed for any particular treatment.

It is important that the half-life of the radionuclide be sufficiently long to allow for its localization in the bone tissue while it still retains sufficient radioactivity to obtain bone marrow suppression. Generally it is preferred to use a radionuclide complex which results in rapid biolocalization of the radionuclide in the bone tissue so as to achieve bone marrow irradiation quickly. It is also beneficial to use a radionuclide having a relatively short half-life so that after bone marrow irradiation is achieved, it is possible to proceed with bone marrow transplantation as soon as possible in order to enhance the prospects of bone marrow engraftment and patient recovery. For example, certain radionuclides such as Sr-89 have been demonstrated, when selectively deposited in bone, to suppress bone marrow [see, for example, Y. Shibata et al., *J. Leukocyte Biol.* 38(6), 659-669 (December 1985)]. However, this compound is not clinically useful since the long half-life of Sr-89 (50 days) prevents transplantation of the new marrow for an unacceptable time. In order to increase the chance of the patient's recovery, it may be beneficial to employ materials, such as granulocyte-macrophage colony stimulating factor, which stimulate or enhance the regeneration of the bone marrow. Radionuclides useful in the method and compositions of this invention are Sm-153, Gd-159, Ho-166, and Y-90, especially preferred is Ho-166.

The radionuclide compositions employed in the method of the present invention are capable of delivering a significant portion of the radioactivity present in the composition to bone tissue rather than to non-target soft tissues. Therefore for those disease states where the treatment regimen requires bone marrow suppression, the present invention is particularly advantageous since it provides a means of achieving selective reduction in the hemopoietic stem cell population without having to resort to total body irradiation, thus resulting in minimal damage to non-target soft tissues. Furthermore, because there is a reduction in the radiation dose delivered to non-target tissues (as compared to the use of total body irradiation), the present invention offers the opportunity to use the same or increased chemotherapeutic dosages. In addition, if it is desirable to employ total body irradiation in conjunction with the bone marrow suppression method described herein, for example, in the treatment of leukemia, it may be possible to reduce the radiation dosage used for the total body irradiation and still obtain the same or higher level of reduction of leukemic cells.

The respective radionuclides can be produced in several ways. In a nuclear reactor, a nuclide is bombarded with neutrons to obtain a nuclide with additional neutrons in its nucleus.

e.g.,

Sm-152 + neutron → Sm-153 + gamma

Typically the desired radionuclide can be prepared by irradiating an appropriate target, such as the metal oxide. Another method of obtaining radionuclides is by bombarding nuclides with particles in a linear accelerator or cyclotron. Yet another way of obtaining radionuclides is to isolate them from fission product mixtures. The method of obtaining the radionuclide is not critical.

Aminophosph nic acids can be prepared by a number of known synthetic techniqu s. Of particular importance is the reaction of a compound containing at least on reactiv amine hydrogen with a carbonyl compound (aldehyde or ketone) phosphorous acid or appropriate derivative thereof, and HCl. Th amine precursor (1,4,7,10-tetraazacyclododecan) employed in making th macrocyclic aminophosphonic acids is a comm rcially available mat rial.

The radionuclide and ligand may be combined under any conditions which allow the two to form a complex. Generally, mixing in water at a controlled pH (the choice of pH is dependent upon the choice of ligand and radionuclide) is all that is required. The complex formed is by a chemical bond and r sults in a relatively stable radionuclid composition, e.g. stable to the disassociation of the radionuclide from th ligand. The preferred bone marrow suppressing radionuclide composition utilizes Ho-166 with DOTMP.

For the purpose of convenience, the radionuclide-macrocyclic aminophosphonic acid compositions will frequently be referred to as "radionuclide compositions" and the macrocyclic aminophosphonic acid derivative referred to as the "ligand" or "chelant".

As used herein, the term "mammal" means a warm blooded mammal, including humans, and is meant to encompass mammals in need of bone marrow suppression, especially humans; thus in some instances the term "patient" is alternatively used for mammal.

The term "bone marrow suppression" refers to a partial or total eradication of the bone marrow, in particular a temporary or permanent reduction of the hemopoietic stem cell population.

For the purpose of the present invention, bone marrow suppressing radionuclide compositions described herein and physiologically acceptable salts thereof are considered equivalent. Physiologically acceptable salts refer to the acid addition salts of those bases which will form a salt with at least one acid group of the ligand or ligands employed and which will not cause significant adverse physiological effects when administered as described herein. Suitable bases include, for example, the alkali metal and alkaline earth metal hydroxides, carbonates, and bicarbonates such as sodium hydroxide, potassium hydroxide, calcium hydroxide, potassium carbonate, sodium bicarbonate, magnesium carbonate, ammonia, primary, secondary and tertiary amines. Physiologically acceptable salts may be prepared by treating the macrocyclic aminophosphonic acid with an appropriate base.

The formulations of the present invention are in the solid or liquid form containing the active radionuclide complexed with the ligand. These formulations may be in kit form such that the two components are mixed at the appropriate time prior to use. Whether premixed or as a kit, the formulations usually require a pharmaceutically acceptable carrier.

Injectable compositions of the present invention may be either in suspension or solution form. In the preparation of suitable formulations it will be recognized that, in general, the water solubility of the salt may be greater than the free acid. In solution form the complex (or when desired the separate components) is dissolved in a physiologically acceptable carrier. Such carriers comprise a suitable solvent, preservatives such as benzyl alcohol, if needed, and buffers. Useful solvents include, for example, water, aqueous alcohols, glycols, and phosphonate or carbonate esters. Such aqueous solutions contain no more than 50% of the organic solvent by volume.

Injectable suspensions as compositions of the present invention require a liquid suspending medium, with or without adjuvants, as a carrier. The suspending medium can be, for example, aqueous polyvinylpyrrolidone, inert oils such as vegetable oils or highly refined mineral oils, or aqueous carboxymethlycellulose. Suitable physiologically acceptable adjuvants, if necessary to keep the complex in suspension, may b chosen from among thickners such as carboxymethylcellulose, polyvinylpyrrolidone, gelatin, and th alginates. Many surfactants are also useful as suspending agents, for example, lecithin, alkylphenol, polyethylene oxide adducts, naphthalenesulfonates, alkylbenzenesulfonates, and the polyoxyethylene sorbitan esters. Many substances which effect the hydrophobicity, density, and surface tension of the liquid suspension medium can assist in making injectable suspensions in individual cases. For example, silicone antifoams, sorbitol, and sugars are all useful suspending agents.

Radionuclide compositions suitable for use in the present invention must have particular properties to be suitable bone marrow suppressing agents. The properties of the particular radionuclide and the particular ligand are important; however, the properties of the combinations of the ligand and radionuclide (that is, th radionuclide compositions) are particularly important. The radionuclide must be taken up preferentially by bone so that it is possible to deliver a bone marrow suppressing dose of radiation to the bone marrow. The radionuclide also should be cleared rapidly from the blood.

The macrocyclic aminophosphonic acid complexes when administered at approximately a ligand to metal molar ratio of 1:1 to 2:1 give biodistributions that are consistent with excellent skeletal agents. By contrast, other aminophosphonic acid complexes result in substantial localization in soft tissue (e.g. liver) if large excess amounts of ligand ar not us d. Excess ligand is und sirable since uncomplexed ligand may be toxic t the pati nt or may r sult in cardiac arr st or hypocalcemic convulsions. In addition, th macrocyclic aminophosphonic acid ligands are useful when large amounts of metal are required (i.e. for m tals that have a low specific activity). In this cas , th macrocyclic aminophosphonic acid ligands have the ability to d posit larger amounts of activity in the bone than is possible wh n using non-cyclic aminophosphonic acid ligands.

The "bone marrow suppressing amount" of radionuclide composition to be administered to achieve bon marrow suppr ssion will vary according to factors such as th age, weight and health of the patient, the disease state being treated, the tr atment regimen selected as well as the nature of the particular radionuclide composition to be administered. For example, less activity will be needed for radionuclides with longer half lives. The energy of the emissions will also be a factor in determining th amount of activity necessary. The preferred range of activity is from about 18 megabecquerels to 1850 megabecquerels per kilogram of body weight of animal to be treated; more preferred is from about 185 megabecquerels to 1850 megabecquerels per kilogram of body weight.

The effective amount used to obtain bone marrow suppression will typically be administered, generally by administration into the bloodstream, in a single dose. The amounts to be administered to achieve bone marrow suppression are readily determined by one skilled in the art employing standard procedures.

As noted previously, the amount of the radionuclide composition used will depend, in part, on the treatment regimen which is selected. For example, in the treatment of a patient having leukemia, the use of the radionuclide compositions described herein can reduce the leukemic cell population in the bone marrow; however, it will usually be necessary to use one or more chemotherapeutic agents, such as dimethyl busulfan and/or cyclophosphamide, to destroy the leukemic cell population in locations other than the bone marrow or in sanctuaries within the bone marrow. In other instances in conjunction with the bon marrow suppression method of the present invention, it may be desirable to employ total body irradiation, with or without chemotherapeutic agents, as a treatment used to reduce the leukemic cell population, such as by delivering radiation to the patient from dual opposing cobalt-60 sources.

The general techniques of bone marrow transplantation are well known in the art, see for example, F. R. Appelbaum et al., "The Role of Marrow Transplantation in the Treatment of Leukemia", (pp. 229-262), C. D. Bloomfield (ed.), Chronic and Acute Leukemias in Adults, 1985, Martinus Nijhoff Publishers, Boston; E. D. Thomas, "Clinical Trials with Bone Marrow Transplantation", (pp. 239-253), Clinical Trials in Cancer Medicine, 1985, Academic Press, Inc.; E. D. Thomas, "Marrow Transplantation for Malignant Diseases", (pp. 517-531), Journal of Clinical Oncology, Vol. 1, No. 9 (September) 1983; E. D. Thomas et al., "Marrow Transplantation for Thalassemia", (pp. 417-427), Annals New York Academy of Sciences, 445, 1985. Under general or spinal anesthesia and using standard marrow aspiration needles, multiple aspirates are performed from the anterior and posterior iliac crests and, occasionally, the sternum of the donor. The marrow is placed in heparinized tissue culture media and then, using metal screens, filtered to remove bony spicules and fat globules and to create a monocellular suspension. At the time of desired administration of the bone marrow, the marrow is infused intravenously, following which the marrow stem cells migrate to the marrow space, proliferate, and eventually restore normal hematopoiesis and immune function. It is probably important to give as many bone marrow cells as possible to enhance the prospects of marrow engraftment. Following the transplant the patient usually receives some form of immunosuppression such as by being administered methotrexate or cyclosporine, in an attempt to prevent or at least modify graft-versus-host disease.

The following examples are included to aid in the understanding of the invention but are not to be construed as limiting the invention.

Example A (Comparative)

Preparation of ethylenediaminetetramethylenephosphonic acid (EDTMP).

Into a suitable reaction vessel equipped with a thermometer, magnetic stirring bar, dropping funnel, and an atmosphere of nitrogen was charged 94.5 grams (g) of phosphorous acid and 100 milliliters (mL) of degassed water. Dissolution of the phosphorous acid was achieved by stirring and then 112 mL of concentrated hydrochloric acid was added. The dropping funnel was charged with 15 g of ethylenediamine and adjusted to allow dropwise addition of the ethylenediamine to the acidic solution. When addition was completed, the solution was refluxed for one hour using a heating mantle. At the end of the one hour reflux period, the dropping funnel was charged with 85 g (37 percent (%) aqueous solution) of formaldehyde which was added dropwise over a two hour period with continued heating to maintain reflux during the addition. After all of the formaldehyde was added, the reaction mixture was stirred under reflux for an additional two hours, th n allowed to cool slowly overnight during which time th product precipitates. Vacuum filtration followed by cold water washing gives ethyl nediaminetetramethylen phosph nic acid (EDTMP).

Example 1

Preparation of 1,4,7,10-tetraazacyclododecanetetramethylenephosphonicacid (DOTMP).

In a 100-mL three necked round-bottomed flask equipped with a thermometer, reflux condenser, and heating mantle was added 3.48 g (20.2 mmole) of 1,4,7,10-tetraazacyclododecane and 14 mL of water. This solution was treated with 17.2 mL of concentrated HCl and 7.2 g of H_3PO_3 (87.8 mmole) and heated to 105°C. The refluxing suspension was stirred vigorously and treated dropwise with 13 g (160.2 mmole) of formaldehyde (37 wt% in water) over a one hour period. At the end of this time the reaction was heated at reflux an additional 2 hours, after which the heat was removed and the reaction solution allowed to cool and set at room temperature (about 20-30°C) for 62.5 hours. The reaction solution was then concentrated *in vacuo* at 40°C to a viscous reddish brown semisolid. A 30 mL portion of water was added to the semisolid which started to dissolve but then began to solidify. The whole suspension was then poured into 400 mL of acetone with vigorously stirring. The resulting off-white precipitate was vacuum filtered and dried overnight to give 10.69 g (97% yield) of crude DOTMP. A 2.0 g (3.65 mmole) sample of the crude DOTMP was dissolved in 2 mL of water by the addition of 700 µL of concentrated ammonium hydroxide (10.0 mmole) in 100 µL portions to give a solution at pH = 2-3. This solution was then added all at once to 4.5 mL of 3N HCl (13.5 mmole), mixed well, and allowed to set. Within one hour small squarish crystals had begun to form on the sides of the glass below the surface of the liquid. The crystal growth was allowed to continue undisturbed for an additional 111 hours after which time the crystals were gently bumped off of the vessel walls, filtered, washed with four 3-mL portions of water, and air dried to constant weight to give 1.19 g (60% yield) of white crystalline solid DOTMP.

Example 2

A 250 mL three-necked, round-bottomed flask was loaded with 6.96 g (0.04 moles) of 1,4,7,10-tetraazacyclododecane. To this flask was added 14.5 g (0.177 moles) of phosphorous acid, 30 mL of deionized water and 28 mL of concentrated hydrochloric acid (0.336 moles).

The flask was attached to a reflux condenser and fitted with a stir bar, and a thermometer adapted with a thermowatch controller. A separate solution of 26.0 g (0.32 moles) of aqueous 37% formaldehyde solution was added to a 100 mL addition funnel and attached to the flask. The flask was brought to reflux temperature (about 105°C) with vigorous stirring. The formaldehyde solution was added dropwise over a 30-40 minute interval. The solution was heated and stirred for an additional three hours then cooled slowly to ambient temperature.

The reaction solution was transferred to a 500 mL round-bottomed flask and attached to a rotary evaporation apparatus. The solution was taken down to a viscous, amber semi-solid (note-temperature never exceeded 40°C). This semi-solid was treated with approximately 300 mL of HPLC grade acetone producing a light brown, sticky viscous oil. This oil was dissolved in 22 mL of water and added slowly with vigorous stirring to 1L of acetone. The acetone was decanted and the light colored oil dried under vacuum to giv 16.6 g (76% yield) of crude DOTMP. To 13.1 g of this crude DOTMP was added 39.3 g of deionized water along with a seed crystal and the solution allowed to stand overnight. The resulting precipitate was vacuum filtered, washed with cold water, and dried under vacuum to give 4.75 g of DOTMP (36% yield).

A further purification was performed by dissolving 3.0 g (5.47 mmole) of DOTMP from above in 3 mL of water by the addition of 2.2 mL (31.5 mmole) of concentrated ammonium hydroxide. This solution was made acidic by the addition of 2.4 mL (28.8 mmole) of concentrated HCl at which time a white solid precipitated. This precipitate was vacuum filtered and dried to give 2.42 g (81% yield) of purified DOTMP characterized by a singlet at 11.5 ppm (relative to 85% H_3PO_4) in the 31p decoupled NMR spectrum.

Example 3

Preparation of ^{153}Sm solution.

Sm-153 can be produced in a reactor such as the University of Missouri Research Reactor. Sm-153 is produc d by irradiating 99.06 perc nt nriched $^{152}Sm_2O_3$ in the first row reflect r at a neutron flux f 8 x 10^{13} neutron/cm²•sec. Irradiations were generally carried out for 50 to 60 hours, yielding a Sm-153 specific activity of 1000-1300 Ci/g.

To irradiate Sm_2O_3 for production of Sm-153, the desired amount of target is first weighed into a quartz vial, the vial flame s aled under vacuum and welded into an aluminum can. Th can is irradiated for the

desired length of time, cooled for several hours and opened remotely in a hot cell. The quartz vial is removed and transferred to a glov box, opened into a glass vial which is then sealed. An appropriate amount of a solution of hydrochloric acid is then added to the vial via syringe in order to dissolve the Sm_2O_3. Once the Sm_2O_3 is dissolved, the samarium solution is diluted to the appropriate volume by addition of water. The solution is removed from the original dissolution vial which contains the chards of th quartz irradiation vial, and transferred via syringe to a clean glass serum vial.

Example 4

Preparation of ^{166}Ho solution.

Holmium-166 is prepared by weighing 0.5-1.0 mg of Ho_2O_3 into a quartz vial. The vial is sealed and placed in an aluminum can which is welded shut. The sample is irradiated (usually for about 24-72 hours) in a reactor (first row reflector, neutron flux of 8 x 10^{13} neutron/cm^2•sec). After irradiation, the vial is opened and the oxide is dissolved using 4 Normal (N) HCl. Heating may be necessary. Water is then used to dilute the sample to an appropriate volume.

Example 5

Preparation of ^{159}Gd solution.

Gadolinium-159 is prepared by sealing gadolinium oxide (1.1 mg) in a quartz vial. The vial is welded inside an aluminum can and irradiated for 30 hours in a reactor at a neutron flux of 8 x 10^{13} neutron/cm^2•sec. The contents of the quartz vial is dissolved using HCl. Water is added to obtain a solution of Gd-159 in 0.1 N HCl.

Example 6

Preparation of ^{90}Y solution.

A commercially available Yttrium-90 solution (Oak Ridge National Laboratories, Oak Ridge, TN) was received as 100 mCi/0.53 mL no carrier added solution of Y-90 as the trichloride in 0.1 N HCl. A nonradioactive YCl_3 solution (0.0003 M) in 0.1 N HCl was prepared. A 700 µL (2.1 x 10^{-7} mol) portion of the nonradioactive YCl_3 solution was added to 45 µL of Y-90 solution to give a final YCl_3 solution at 2.82 x 10^{-4} M containing Y-90.

Example B (Comparative)

Preparation of ^{153}Sm-EDTMP.

A solution of 0.3 mM Sm in 0.1N HCl was spiked with Sm-153. Three mL of this solution was transferred to a vial containing a freeze dried solution of NaOH and ethylenediaminetetramethylenephosphonic acid (EDTMP). The resultant concentration of EDTMP was 35 mg/mL and the pH was between 7 and 8. Lower ligand to metal ratios were obtained by diluting the stock Sm-EDTMP solution with Sm solution and adjusting the pH to 7-8. The amount of metal found as a complex was determined by cation exchange chromatography to be >99% for all the solutions.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the Sm-EDTMP solutions via a tail vein. The rats weighed between 150 and 200 g at the time of injection. Aft r 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts were compared to the counts in 100 µL standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in liver for formulations at various EDTMP to Sm molar ratios are given in Table I. The numbers represent the averag of 3 rats per data point.

TABLE I

% INJECTED DOSE IN LIVER AT VARIOUS EDTMP TO Sm RATIOS		
EDTMP[1]	L:M[2]	% Dose in Liver
0.002	8	1.8
0.006	19	0.64
0.011	38	0.33
0.023	76	0.17
0.046	153	0.15

[1] EDTMP is in moles per liter.
[2] L:M = The ligand to Sm molar ratios

Example C (Comparative)

Preparation of ^{166}Ho-EDTMP.

A solution of 0.6 mM Ho in 0.1N HCl was spiked with Ho-166. Three mL of this solution was transferred to a vial containing a freeze dried solution of NaOH and ethylenediaminetetramethylenephosphonic acid (EDTMP). The resultant concentration of EDTMP was 35 mg/mL and the pH was between 7 and 8. Lower ligand to metal ratios were obtained by diluting the stock Ho-EDTMP solution with Ho solution and adjusting the pH to 7-8. The amount of metal found as a complex was determined by cation exchange chromatography to be >99% for all the solutions.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the Ho-EDTMP solutions via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts were compared to the counts in 100 µL standards in order to determined the percentage of the dose in each tissue or organ. The percent of the injected dose in liver for formulations at various EDTMP to Ho molar ratios are given in Table II. The numbers represent the average of 5 rats per data point.

TABLE II

% INJECTED DOSE IN LIVER AT VARIOUS EDTMP TO Ho RATIOS		
EDTMP[1]	L:M[2]	% Dose in Liver
0.042	70	0.07
0.030	50	0.08
0.024	39	0.07
0.018	30	0.10
0.012	20	0.17
0.006	10	0.79
0.003	5	0.94

[1] EDTMP is in moles/lit r.
[2] L:M = ligand to Ho molar ratio

Example 7

Preparation of ^{153}Sm-DOTMP.

Th ligand of Example 1 (22 mg) was dissolved in 878 µL of distilled water and 15 µL of 50% NaOH. A volume of 15 µL of this solution was transferred to a vial containing 1.5 mL of Sm solution (0.3 mM Sm in 0.1N HCl spiked with 2 µL of Sm-153 tracer). The pH was adjusted to 7-8 using NaOH and the amount of Sm found as a complex was greater than 99% as determined by ion exchange chromatography. This yielded a solution containing Sm at 0.3 mM with a ligand to metal molar ratio of approximately 1.5.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the Sm solution described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts were compared to the counts in 100 µL standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table III. The numbers represent the average of 3 rats per data point.

TABLE III

% INJECTED DOSE IN SEVERAL TISSUES FOR Sm-DOTMP[1]	
Bone	58.1
Liver	0.06
Kidney	0.27
Spleen	0.004
Muscle	0.15
Blood	0.004

[1] Ligand to Sm Molar Ratio of approximately 1.5

Example 8

Preparation of ^{166}Ho-DOTMP.

The ligand of Example 1 (22 mg) was dissolved in 878 µL of distilled water and 15 µL of 50% NaOH. A volume of 30 µL of this solution was transferred to a vial containing 1.5 mL of Ho solution (0.6 mM Ho in 0.1N HCl spiked with 2 µL of Ho-166 tracer). The pH was adjusted to 7-8 using NaOH and the amount of Ho found as a complex was greater than 99% as determined by ion exchange chromatography. This yielded a solution containing 0.6 mM Ho with a ligand to metal molar ratio of approximately 1.5.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the Ho solution described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts were compared to the counts in 100 µL standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table IV. The numbers represent the average of 3 rats per data point.

TABLE IV

% INJECTED DOSE IN SEVERAL TISSUES FOR Ho-DOTMP[1]	
Bone	57
Liver	0.07
Kidney	0.4
Spleen	0.006
Muscle	0.3
Blood	0.07

[1] Ligand to Ho Molar Ratio of approximately 1.5

Example 9

Preparation of ^{153}Sm-DOTMP and ^{166}Ho-DOTMP.

A quantity of 14.5 mg of the ligand of Example 2 was placed in a vial and dissolved in 760 µL of water and 5 µL of 50% NaOH. A volume of 1100 µL of Sm solution (0.3 mM Sm in 0.1 N HCl) which was spiked with Sm-153, was placed in a separate vial and 10 µL of the ligand solution was added. The pH of the solution was adjusted to 7-8 using NaOH and the solution was passed through 3 plastic columns containing 1.5 mL of cation exchange resin (Sephadex C-25 from Pharmacia). The amount of Sm as a complex was determined to be 99% by cation exchange chromatography.

A volume of 1100 µL of Ho solution (0.6 mM Ho in 0.1 N HCl) which was spiked with Ho-166, was placed in a separate vial and 20 µL of the above ligand solution was added. The pH of the solution was adjusted to 7-8 using NaOH and the solution was passed through 2 plastic columns containing 1.5 mL of cation exchange resin (Sephadex C-25 from Pharmacia). The amount of Ho as a complex was determined to be 99% by cation exchange chromatography.

Sprague Dawley rats were allowed to acclimate for five days then injected with 100 µL of the solutions described above via a tail vein. The rats weighed between 150 and 200 g at the time of injection. After 2 hours the rats were killed by cervical dislocation. Tissues were taken, weighed and the amount of radioactivity determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. Th counts in each tissue were compared to the counts in 100 µL standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues ar given in Table V. The numbers represent the average of 3 rats per data point.

TABLE V

% INJECTED DOSE IN SEVERAL TISSUES FOR DOTMP METAL COMPLEXES		
	Sm	Ho
Bone	50	64
Liver	0.37	0.19
Kidney	0.29	0.32
Spleen	0.04	0.05
Muscle	0.49	0.22
Blood	0.12	0.17

Example 10

Preparation of ^{166}Ho-DOTMP.

A volume of 0.5 mL of non-radioactive holmium solution (0.6 mM) in 0.1N HCl was mixed with 0.5 mL of Ho-166 solution (also 0.6 mM in Ho, dissolved in 0.1N HCl) in a plastic vial. To this was added 30 µL of a 33 mM aqueous solution of the ligand of Example 2. Sodium hydroxide (50%) was added slowly until the pH was between 7 and 8. The percentage of the total Ho found as a complex was determined to be greater than 99% by cation exchange chromatography.

Six Sprague Dawley rats were allowed to acclimate for a period of 6 days then a sample of blood was taken daily from the tail vein and the white blood cell count determined by a standard manual method (Unopette Test 5856 from Becton-Dickinson and Company). On the fourth day, rats numbered 2, 4 and 6 were injected with 0.9 mCi (33.3 MBq) of the above complex. The rat weight at this time ranged from 160-180 g. On days 7, 8, and 9 the white blood cell count was again determined for each rat. Table VI gives the white blood cell count of the injected (2, 4 and 6) rats compared to the control rats (1, 3, and 5). There is a significant drop in the blood count of the treated animals compared to the untreated animals.

TABLE VI

WHITE BLOOD CELL COUNTS **							
Rat[1]	Day 1	Day 2	Day 3	Day 4*	Day 7	Day 8	Day 9
1	17650	16500	18550	19950	20550	19750	21500
2	16550	15850	19900	28950	5850	7950	7900
3	21650	19250	20300	20550	22700	26550	26050
4	20900	20300	21500	21300	7400	7500	7550
5	20650	19400	20250	20950	18700	17700	23750
6	20250	18400	17450	17600	5400	4750	5350

*Date of injection

[1]Rats 1, 3 and 5 are control; Rats 2, 4 and 6 were injected with Ho-DOTMP.

** cells/cubic millimeter (mm^3)

Example 11

Preparation of ^{159}Gd-DOTMP.

The ligand of Example 2 (14.5 mg) was placed in a vial and dissolved in 760 µL of water and 5 µL of 50% NaOH. A volume of 1000 µL of Gd solution (0.3 mM Gd in 0.1 N HCl) which contained tracer quantities of Gd-159, was placed in a separate vial and 15 µL of the ligand solution was added. The pH of the solution was adjusted to 7-8 using NaOH and the amount of Gd as a complex was determined to be >99% by cation exchange chromatography.

A Sprague Dawley rat was allowed to acclimate for five days then injected with 175 µL of the solution described above via a tail vein. The rat weighed 155 g at the time of injection. After 2 hours the rat was killed by cervical dislocation and dissected. The amount of radioactivity in each tissue was determined by counting in a NaI scintillation counter coupled to a multichannel analyzer. The counts in each tissue were compared to the counts in 175 µL standards in order to determine the percentage of the dose in each tissue or organ. The percent of the injected dose in several tissues are given in Table VII.

TABLE VII

% INJECTED DOSE IN SEVERAL TISSUES FOR Gd-DOTMP	
Tissue	% Dose
Bone	50
Liver	0.08
Kidney	0.25
Spleen	None detected*
Muscle	0.08
Blood	0.06

* Counts in the spleen were below background

Example 12

Preparation of ^{90}Y-DOTMP.

A 0.0015 M solution of the ligand of Example 2 was prepared and a 22.5 µL (3.3×10^{-8} mol) portion was added to 745 µL (2.1×10^{-7} mol) of Y-90 solution from Example 6. Sodium hydroxide (50%) was added slowly until the pH was 7.5. A 10.0 µL addition of the 100 mCi/0.53 mL no carrier added solution of Y-90 (described in Example 6) was then added to bring the specific activity to 1.0 mCi/100 µL. The percentage of the total Y found as a complex was determined to be greater than 99% by cation exchange chromatography.

Six Sprague Dawley rats were allowed to acclimate for a period of 6 days then a sample of blood was taken from the tail vein (day = -7) and the white blood cell count determined by a standard manual method (Unopette Test 5856 from Becton-Dickinson and Company). Three days later (day = -4) this procedure was repeated. Four days later (day = 0) the procedure was again performed and rats 3, 5 and 6 were injected in the tail vein with 100 µL of the above ^{90}Y-DOTMP complex. These injections contained approximately 1 mCi (37.0 MBq) of the above Y-90 complex each. On days 3 and 5 the white blood cell count was again determined for all 6 rats. Table VIII gives the white blood cell count of the injected (3, 5 and 6) rats compared to the control rats (1, 2 and 4). There is a significant drop in the blood count of the treated animals compared to the untreated animals.

TABLE VIII

WHITE BLOOD CELL COUNTS **					
Rat¹	Day -7	Day -4	Day 0*	Day 3	Day 5
1	13600	19200	16100	15000	19200
2	23000	20800	15400	19200	28400
3	21800	15000	16400	4000	2500
4	21600	22200	16800	18600	18600
5	14000	20800	13000	6250	3400
6	15600	18000	13650	5550	4200

*Dat of injection

¹Rats 1, 2 and 4 are control; Rats 3, 5 and 6 were injected with Y-DOTMP.

** cells/cubic millimeter (mm³)

Claims

1. A steril composition for suppressing bone marrow in a mammal comprising at l ast one radionuclid selected from Samarium-153, Gadolinium-159, Holmium-166 or Yttrium-90 complexed with at least one macrocyclic aminophosphonic acid ligand containing th 1,4,7,10-t traazacyclododecan moiety or a physiologically-acceptable salt thereof, wherein the phosphorous of said ligand is attached to the amine nitrogen through an alkylene moiety and wherein the radionuclide in dosage form is present in an amount containing from 18 to 1850 megabecquerels per kilogram of body weight of said mammal.

2. The composition of Claim 1 wherein the dosage form contains from 185 to 1850 megabecquerels per kilogram of body weight of said mammal.

3. The composition of Claim 1 or 2 wherein the radionuclide is Samarium-153.

4. The composition of Claim 1 or 2 wherein the radionuclide is Gadolinium-159.

5. The composition of Claim 1 or 2 wherein the radionuclide is Holmium-166.

6. The composition of Claim 1 or 2 wherein the radionuclide is Yttrium-90.

7. The composition as claimed in any one of Claims 1 to 6 wherein the ligand to radionuclide ratio is from 1:1 to 2:1.

8. A pharmaceutical formulation comprising the composition as claimed in any one of Claims 1 to 7 in a pharmaceutically acceptable carrier.

Patentansprüche

1. Sterile Zusammensetzung zur Knochenmarkunterdrückung in einem Säuger, umfassend wenigstens ein Radionuclid, ausgewählt aus Samarium-153, Gadolinium-159, Holmium-166 oder Yttrium-90, welch s mit wenigstens einem makrocyclischen Aminophosphonsäureliganden komplexiert ist, welcher den 1,4,7,10-Tetraazacyclododecanrest oder ein physiologisch verträgliches Salz davon enthält, worin der Phosphor des Liganden mit dem Aminstickstoff durch einen Alkylenrest verbunden ist und worin das Radionuclid in Dosisform in einer Menge vorliegt, die 18 bis 1850 Megabecquerel pro Kilogramm Körpergewicht des Säugers enthält.

2. Zusammensetzung nach Anspruch 1, worin die Dosisform 185 bis 1850 Megabecquerel pro Kilogramm Körpergewicht des Säugers enthält.

3. Zusammensetzung nach Anspruch 1 oder 2, worin das Radionuclid Samarium-153 ist.

4. Zusammensetzung nach Anspruch 1 oder 2, worin das Radionuclid Gadolinium-159 ist.

5. Zusammensetzung nach Anspruch 1 oder 2, worin das Radionuclid Holmium-166 ist.

6. Zusammensetzung nach Anspruch 1 oder 2, worin das Radionuclid Yttrium-90 ist.

7. Zusammensetzung nach einem der Ansprüche 1 bis 6, worin das Verhältnis von Ligand zu Radionuclid 1 : 1 bis 2 : 1 ist.

8. Pharmazeutische Formulierung, umfassend die Zusammensetzung nach einem der Ansprüche 1 bis 7 in einem pharmazeutisch verträglichen Träger.

Revendications

1. Composition stérile pour supprim r la mo lle oss use chez un mammifère, comprenant au moins un radionucléide choisi parmi le samarium-153, le gadolinium-159, le holmium-166 et l'yttrium-90, complexé avec au moins un ligand macrocyclique de type acide aminophosphonique, comportant un

fragment 1,4,7,10-tétraaza-cyclododécan , ou un de ses sels physiologiquem nt acceptables, les atomes de phosphore dudit ligand étant rattachés aux atomes d'azote de type amine par l'intermédiaire d'un chaînon alkylèn , et le radionucléide étant présent, dans la forme posologiqu , en une quantité représentant de 18 à 1850 mégabecquerels par kilogramme de poids corporel dudit mammifère.

2. Composition conforme à la revendication 1, dans laquelle la forme posologique contient de 185 à 1850 mégabecquerels par kilogramme de poids corporel dudit mammifére.

3. Composition conforme à la revendication 1 ou 2, dans laquelle le radionucléide est du samarium-153.

4. Composition conforme à la revendication 1 ou 2, dans laquelle le radionucléide est du gadolinium-159.

5. Composition conforme à la revendication 1 ou 2, dans laquelle le radionucléide est du holmium-166.

6. Composition conforme à la revendication 1 ou 2, dans laquelle le radionucléide est de l'yttrium-90.

7. Composition conforme à l'une quelconque des revendications 1 à 6, dans laquelle le rapport du ligand au radionucléide vaut de 1/1 à 2/1.

8. Formulation pharmaceutique comprenant une composition conforme à l'une quelconque des revendications 1 à 7, dans un véhicule pharmaceutiquement acceptable.